

# Circulating Monocytes and In-Stent Neointima After Coronary Stent Implantation

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<b>OBJECTIVES</b>	The aim of this study was to investigate the relationship between circulating monocytes and in-stent neointimal volume at six-month follow-up.
<b>BACKGROUND</b>	In-stent neointimal hyperplasia is the main contributing factor to in-stent restenosis. There is increasing evidence that white blood cells (WBCs), especially monocytes, play a central role in restenosis after stent implantation.
<b>METHODS</b>	We performed coronary stent implantation in 107 patients (107 lesions). Peripheral blood was obtained from all patients immediately before coronary angiography and every day for seven days after the intervention, and each WBC fraction count was analyzed. At scheduled six-month follow-up, all patients received angiographic and volumetric intravascular ultrasound analysis.
<b>RESULTS</b>	The circulating monocyte count increased and reached its peak two days after stent implantation (from $350 \pm 167$ to $515 \pm 149/\text{mm}^3$ , $p < 0.01$ ). The maximum monocyte count after stent implantation showed a significant positive correlation with in-stent neointimal volume at six-month follow-up ( $r = 0.44$ , $p < 0.0001$ ). Other fractions showed neither significant serial changes nor a correlation with in-stent neointimal volume. Multiple regression analysis revealed that in-stent neointimal volume was independently correlated with stent volume immediately after implantation ( $r = 0.45$ , $p < 0.0001$ ) and maximum monocyte count ( $r = 0.35$ , $p < 0.001$ ). Angiographic restenosis, defined as percent diameter stenosis $>50\%$ , was observed in 22 patients (21%), and these patients showed a significantly larger maximum monocyte count than patients without restenosis ( $642 \pm 110$ vs. $529 \pm 77/\text{mm}^3$ , $p < 0.01$ ).
<b>CONCLUSIONS</b>	Circulating monocytes increased after coronary stent implantation, and the peak monocyte count related to in-stent neointimal volume. Our results suggest that circulating monocytes play a role in the process of in-stent neointimal hyperplasia. (J Am Coll Cardiol 2004;43:18–23) © 2004 by the American College of Cardiology Foundation

Stent implantation for coronary artery disease is now established as a therapeutic strategy of great benefit (1,2). However, in-stent restenosis, which is a main limitation of coronary stent implantation, remains unresolved (3).

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Excessive in-stent neointimal hyperplasia is the main contributing factor to in-stent restenosis (4,5). There is increasing evidence from several experimental studies that white blood cells (WBCs), especially monocytes, play a central role in restenosis after balloon angioplasty and stent implantation (6–9). Previous studies have demonstrated infiltration and accumulation of monocytes to the stenting site, and these cells correlate with smooth muscle cell (SMC) proliferation and neointimal growth (6,7). Also, monocytes/macrophages have been demonstrated to be one of the components of the neointima (9). However, there are few studies that investigate the relationship between circu-

lating monocytes and in-stent neointima of human stented lesions.

The aim of this study was to investigate the relationship between circulating monocytes and in-stent neointimal volume at six-month follow-up. We monitored the serial changes in the monocyte count after stent implantation and evaluated the relationship with in-stent neointimal volume.

## METHODS

**Patient enrollment.** Our patient population comprised 107 consecutive patients (107 lesions) who received successful intravascular ultrasound (IVUS)-guided single-stent (stent length 15 mm) implantation between May 2001 and March 2002 and returned for scheduled six-month follow-up analysis. In this study, patients who met the following criteria were excluded: 1) patients with a restenotic lesion from a previous intervention; 2) patients requiring multiple stenting in one lesion; 3) patients having a culprit lesion with an arc of calcification  $\geq 90^\circ$ ; and 4) patients with a left main coronary artery lesion or chronic total occlusion lesion.

To examine the effect of coronary angiography itself on each WBC fraction count, we selected 131 patients who underwent coronary angiography but had no intervention in the same period as a non-intervention group.

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# Abbreviations and Acronyms

CSA	= cross-sectional area
EEM	= external elastic membrane
IVUS	= intravascular ultrasound
SMC	= smooth muscle cell
WBC	= white blood cell

**Protocol for intervention and follow-up.** Coronary angiography was performed using a standard technique (10). All patients initially received a bolus injection of 10,000 IU heparin and intracoronary isosorbide dinitrate (5 mg) before angiography. After completion of the diagnostic coronary angiogram, pre-intervention IVUS was performed using a 0.014-inch (0.035-cm) guide wire. The IVUS catheter (3.2F; Ultra Cross, Boston Scientific Scimed, Inc., Maple Grove, Minnesota) was carefully advanced distal to the lesion under fluoroscopic guidance. It was then pulled back automatically from the distal portion at 0.5 mm/s to the culprit lesion, and the vessel diameter (media to media) was measured. All IVUS images were recorded on S-VHS videotape for off-line analysis. After IVUS evaluation, balloon pre-dilation and primary stenting were performed using a Multi-Link stent (ACS, Santa Clara, California) sized to 90% of the media-to-media diameter on IVUS and dilated at 14 atm. After stent implantation, a non-compliant balloon, sized in the same way, was used for post-dilation at  $\geq 10$  atm, with the inflation pressure increased until the minimum lumen area of the stented site was  $\geq 80\%$  of the distal reference

**Table 1.** Patient Characteristics and Angiographic Results

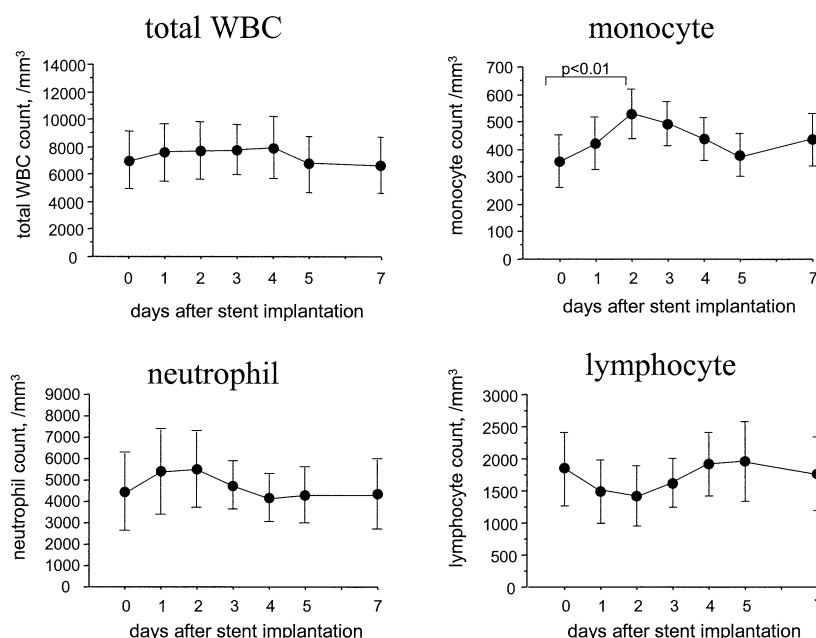
No. of patients (lesions)	107 (107)
Age (yrs)	63.8 $\pm$ 9.2
Male/female	82 (77%)/25 (23%)
Hypertension	51 (48%)
Diabetes mellitus	31 (29%)
Hypercholesterolemia ( $>220$ mg/dl)	47 (44%)
Smoking	46 (43%)
Reference diameter (mm)	3.0 $\pm$ 0.3
Minimum lumen diameter (mm)	0.5 $\pm$ 0.4
Diameter stenosis (%)	83.4 $\pm$ 14.0
Stent size (mm)	3.5 $\pm$ 0.3
Maximum inflation pressure (atm)	12.2 $\pm$ 1.4
Final reference diameter (mm)	3.3 $\pm$ 0.3
Final minimum lumen diameter (mm)	2.9 $\pm$ 0.4
Final diameter stenosis (%)	11.6 $\pm$ 6.1

Data are presented as mean  $\pm$  SD or number (%) of patients.

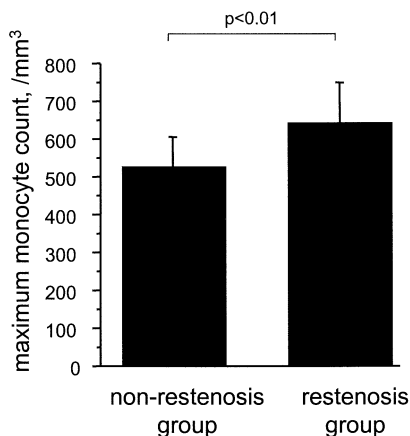
cross-sectional area (CSA) on IVUS. All patients were started on a post-procedural regimen of 81 mg/day aspirin and 200 mg/day ticlopidine hydrochloride.

At scheduled six-month follow-up, patients received a bolus injection of 3,000 IU heparin and 5 mg intracoronary isosorbide dinitrate, with coronary angiography and volumetric IVUS analysis using the same protocol. The protocol for the study was approved by the Ethics Committee of Osaka City University Hospital. We obtained written, informed consent from all participants before coronary angiography and stent implantation.

**Measurement of WBC count.** Peripheral blood was obtained from all patients immediately before coronary angiography and every day for seven days after the procedure. Blood sampling was performed carefully and gently to avoid



**Figure 1.** Serial changes in each white blood cell (WBC) fraction count after stent implantation were demonstrated. Monocytes increased after stent implantation and reached a peak level two days after the procedure (from  $350 \pm 167$  to  $515 \pm 149/\text{mm}^3$ ,  $p < 0.01$ ). Other fractions had no significant serial change.



**Figure 2.** Comparison of maximum monocyte counts. Patients with restenosis showed a significantly larger maximum monocyte count than did patients without restenosis ( $642 \pm 110$  vs.  $529 \pm 77/\text{mm}^3$ ,  $p < 0.001$ ).

hemolysis. Total WBCs and each fraction count were measured with an automated hematology analyzer (XE-2100, Sysmex, Kobe, Japan). Measurements of WBC count were also made using the same protocol for patients in the non-intervention group.

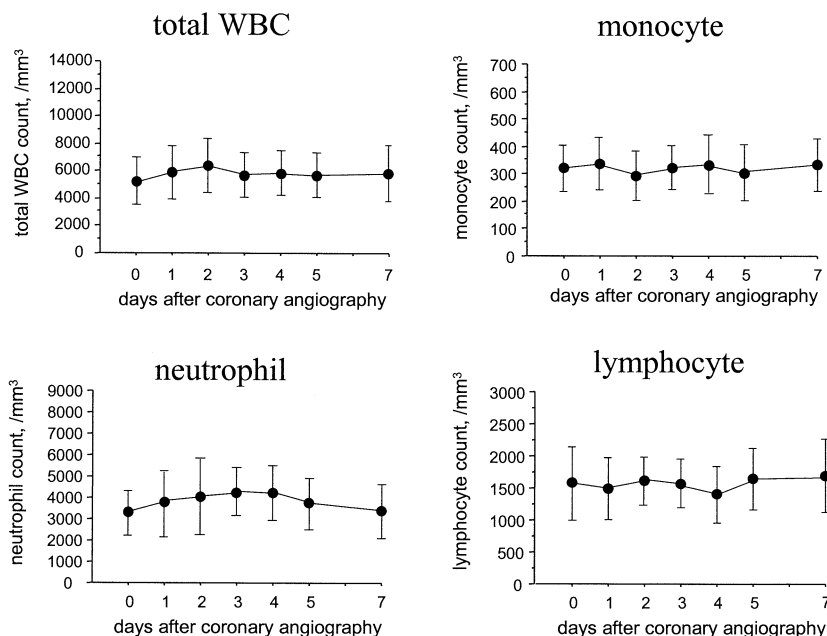
**Angiographic analysis.** Coronary angiograms were reviewed separately by two independent observers unaware of the IVUS findings and clinical data (Drs. Shimada and Yoshiyama). Quantitative angiography was performed off-line using the CMS-QCA system (CMS-MEDIS, Medical Imaging Systems, Leiden, The Netherlands). Reference diameter, minimum lumen diameter, and percent diameter stenosis at the end-diastolic phase were calculated. Angiographic restenosis was defined as percent diameter stenosis  $>50\%$  at follow-up.

**Volumetric IVUS analysis.** Videotaped IVUS images were analyzed by two experienced observers (Drs. Tanaka and Kawarabayashi) blinded to the angiograms and clinical results. Measurements of the external elastic membrane (EEM) CSA, stent area, and lumen area were made every 2 s of videotape, using a computer-associated algorithm (TapeMeasure, Indec Systems, Capitola, California). Therefore, in effect, each stent was axially divided into several 1-mm segments. Volumetric measurement of the stented segment was made by applying Simpson's rule. Neointimal volume was defined as the difference between stent volume and lumen volume. The reproducibility of IVUS measurements of EEM, stent area, and lumen area has already been reported. In no patient was visualization of the EEM in stented segments hampered by the stent filaments.

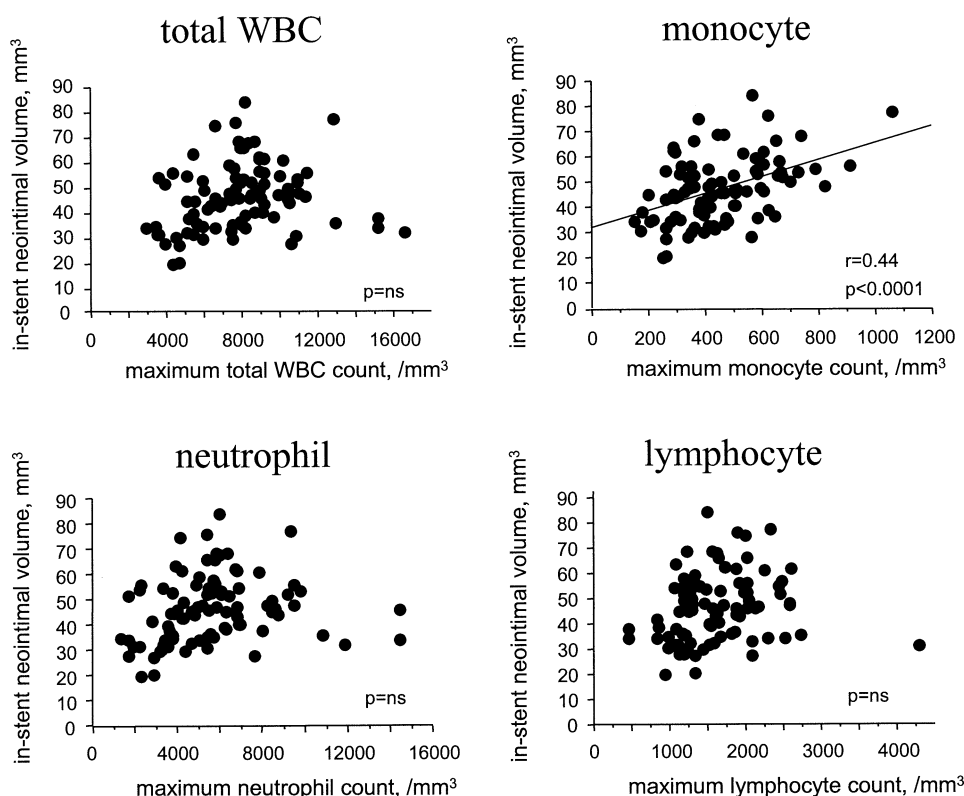
**Statistical analysis.** Data are expressed as the mean value  $\pm$  SD. Serial changes in each WBC fraction count were evaluated by two-way analysis of variance with repeated measures using the Scheffé F test. The strength of the association of each WBC fraction count with in-stent neointimal volume was assessed by linear regression analysis. Multiple regression analysis was performed for several parameters (i.e., male gender, age, classic coronary risk factors, stent volume immediately after stent implantation, and maximum monocyte count), predicting the in-stent neointimal volume. A  $p$  value  $<0.05$  was considered statistically significant.

## RESULTS

**Patient characteristics.** Baseline patient characteristics are shown in Table 1. In our study population, there was



**Figure 3.** Serial changes in each white blood cell (WBC) fraction count in the non-intervention group were demonstrated. Unlike patients who underwent stent implantation, there were no significant serial changes in each WBC fraction count, including monocytes, in the non-intervention group.



**Figure 4.** The relationship between the maximum count of each white blood cell (WBC) fraction and in-stent neointimal volume after six-month follow-up was demonstrated. The maximum monocyte count showed a significant positive correlation with in-stent neointimal volume ( $r = 0.44$ ,  $p < 0.0001$ ). Other fractions had no significant correlation with in-stent neointimal volume.

no in-hospital death or subacute stent thrombosis, and during the follow-up period, there were also no major adverse cardiac events. Follow-up angiography and IVUS evaluation were performed at  $6.5 \pm 1.1$  months in all patients (107 lesions). At follow-up, angiographic in-stent restenosis was observed in 22 patients (21%).

**Peripheral monocyte count.** The serial changes in each WBC fraction count are shown in Figure 1. The monocyte count increased after stent implantation and reached its peak two days after the procedure (from  $350 \pm 167$  to  $515 \pm 149/\text{mm}^3$ ,  $p < 0.01$ ). However, other fractions had no significant change. Also, patients with restenosis showed a significantly larger maximum monocyte count than did patients without restenosis ( $642 \pm 110$  vs.  $529 \pm 77/\text{mm}^3$ ,  $p < 0.001$ ), as shown in Figure 2. Patients in the non-intervention group showed no significant serial changes in each WBC fraction count, including monocytes, as shown in Figure 3.

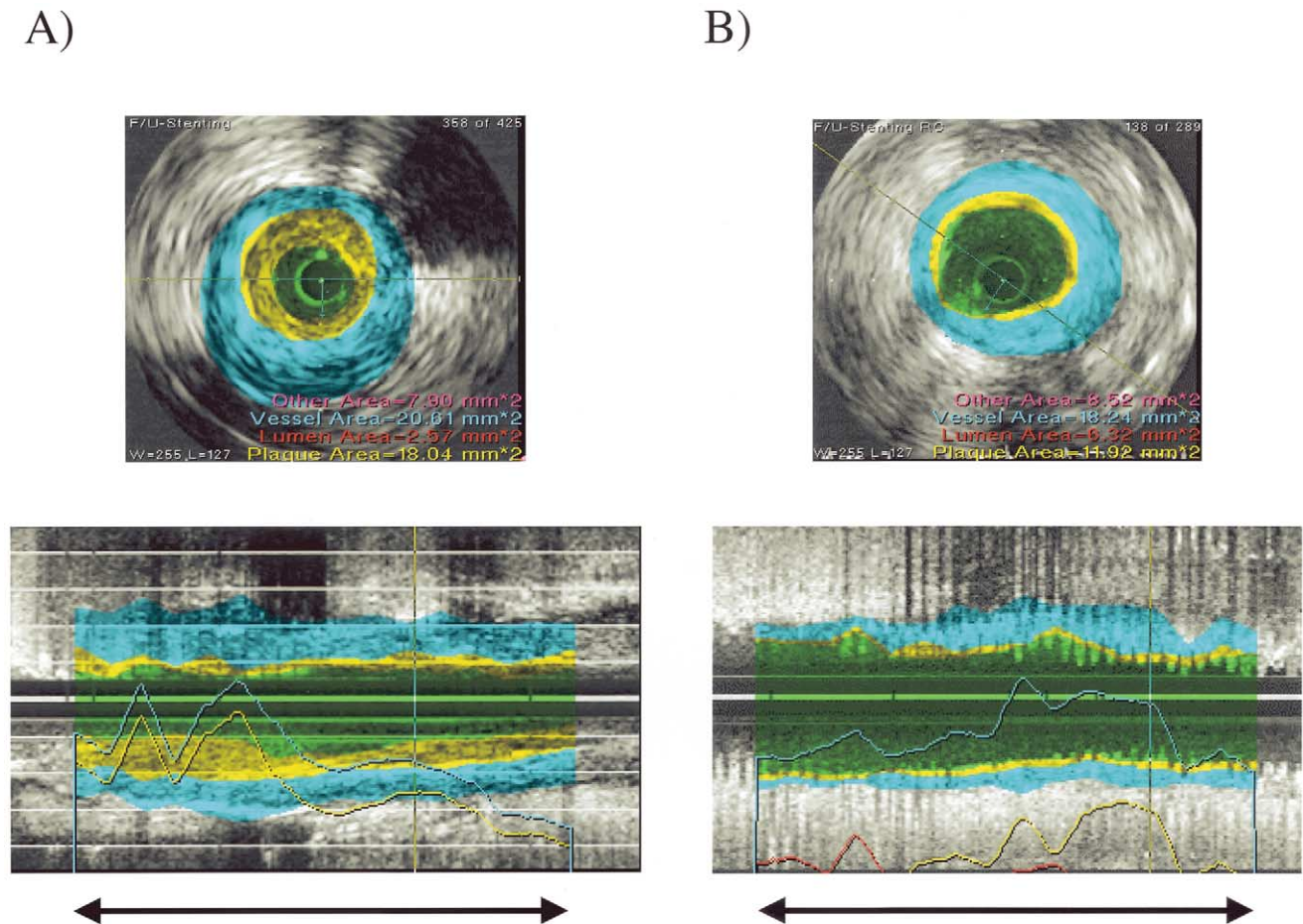
**Relationship between monocyte count and in-stent neointimal volume.** The maximum monocyte count after stent implantation showed a significant positive correlation with in-stent neointimal volume at six-month follow-up ( $r = 0.44$ ,  $p < 0.0001$ ), as shown in Figure 4. However, other fractions had no correlation with in-stent neointimal volume. Multiple regression analysis revealed that in-stent neointimal volume was independently correlated with stent

volume immediately after stent implantation ( $r = 0.45$ ,  $p < 0.0001$ ) and maximum monocyte count ( $r = 0.35$ ,  $p < 0.001$ ), as shown in Table 2.

Figure 5 shows typical cases of patients with restenosis (part A) and without restenosis (part B). Both of these patients underwent stent implantation in the mid right coronary artery portion using a 3.5-mm Multi-Link stent (stent length 15 mm). One patient (Fig. 5A) presented with  $76.12 \text{ mm}^3$  of in-stent neointima and  $621/\text{mm}^3$  maximum monocyte count, whereas another patient (Fig. 5B) had  $27.9 \text{ mm}^3$  of in-stent neointima and  $259/\text{mm}^3$  maximum monocyte count.

**Table 2.** Multiple Regression Analysis for Predicting In-Stent Neointimal Volume

	Standard Regression Coefficient	p Value
Age (yrs)	0.052	0.572
Male	−0.003	0.977
Hypertension	0.009	0.919
Diabetes mellitus	0.074	0.41
Hypercholesterolemia	0.122	0.171
Stent volume immediately after implantation	0.423	< 0.0001
Maximum monocyte count ( $/\text{mm}^3$ )	0.324	0.0007



**Figure 5.** Typical examples of patients with restenosis (A) and without restenosis (B). Both of these patients underwent stent implantation in the mid right coronary artery portion using a 3.5-mm Multi-Link stent (15 mm in length) (arrows). The patient with restenosis presented with 76.12 mm<sup>3</sup> of in-stent neointima and 621/mm<sup>3</sup> maximum monocyte count, whereas the patient without restenosis had 27.9 mm<sup>3</sup> of in-stent neointima and 259/mm<sup>3</sup> maximum monocyte count.

## DISCUSSION

Previously, the important role of WBCs, especially monocytes, in in-stent neointimal proliferation has been demonstrated, although no study has revealed the relationship between in-stent neointima and circulating monocytes. We showed the positive correlation between circulating monocytes and in-stent neointimal volume in human stented lesions. The SMCs are known to be the major cellular component of in-stent neointima. Excessive accumulation of SMCs has a key role in in-stent neointimal hyperplasia; however, the origin of SMCs is still not well established.

Neointimal SMCs were thought to originate locally from the SMCs of the medial layer in the vascular injury models and graft vasculopathy models (11,12). Adherent activated monocytes/macrophages secrete numerous growth factors, cytokines, and metalloproteinases and promote the migration and proliferation of SMCs to the subendothelial space (13–15). Our results may reflect the monocyte-related migration and proliferation of SMCs and neointimal growth.

The advance of stem-cell science has revealed that bone marrow contains multipotent adult stem cells with a high capacity for differentiation (16–20). The peripheral monocyte fraction, as we focused on in this study, is known to include bone marrow stem cells (21,22). Previous reports have demonstrated that neointimal lesions in graft vasculopathy models were formed by the attachment and proliferation of recipient circulating SMC progenitor cells, and these reports have also demonstrated that bone marrow cells contribute to neointimal lesions in vascular injury models (23,24). Also, other reports have demonstrated that circulating endothelial progenitor cells derived from bone marrow incorporate into re-endothelialization after vascular injury (25,26). A more recent study has reported the outgrowth of SMCs from peripheral circulating mononuclear cells (27). Our results suggest a possible mechanism by which increased monocytes, including progenitor cells, derived from bone marrow, differentiate to the neointimal SMCs and contribute to neointimal formation in human stented lesions.

The origin of SMCs is still controversial, and further

studies are needed. In either case, circulating monocytes may play an important role in the process of neointimal proliferation after stent implantation in humans.

**Study limitations.** Our study has several limitations. We did not investigate membrane antigen of monocytes, so the exact origin of increasing monocytes after stent implantation was unclear. In addition, functional analysis of monocytes was not performed. Finally, to analyze each WBC fraction count, we only performed automated measurements in this study.

**Clinical implication.** Our results suggest that circulating monocytes relate to the process of in-stent neointimal hyperplasia in human stented lesions. This proposes a new point of view of mechanisms of in-stent restenosis and suggests a new therapeutic target.

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